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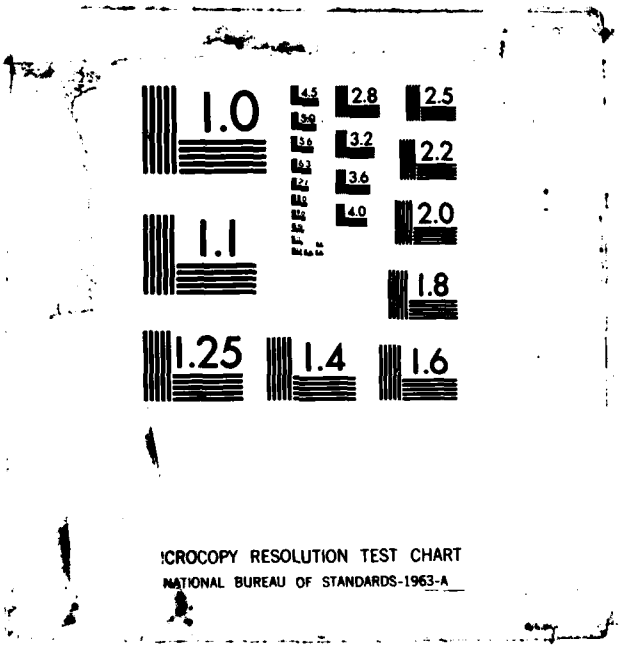
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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF
AI3-37135 IN ANIMALS
STUDY NO. 75-51-0532-86
MARCH 1985 - JULY 1986

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Preliminary toxicity data are presented for the candidate cockroach repellent AI3-37135. These data are intended to provide guidance in selecting compounds for further entomological and toxicological evaluation. The rat oral approximate lethal dose was 2222 mg/kg. This compound was also found to produce moderate to severe skin and eye irritation in rabbits. It was not a sensitizer in guinea pigs and was negative in the Ames test for mutagenicity. AI3-37135 was not an inhalation hazard at room temperature, however, when heated, its vapors may reach high enough concentrations to cause skin, eye and respiratory irritation. When administered to anesthetized rats, it produced physiological changes which suggest the cardiovascular system as a potential site of toxic action.

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ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO ATTENTION OF

HSHB-MO-T

30 September 1986

SUBJECT: Preliminary Assessment of the Relative Toxicity of AI3-37135 in Animals, Study No. 75-51-0532-86, March 1985 - July 1986

Executive Director
Armed Forces Pest Management Board
Forest Glen Section, WRAMC
Washington, DC 20307-5001

EXECUTIVE SUMMARY

The purpose and a summary of the recommendations of the enclosed report follow:

a. Purpose. To provide preliminary toxicity data for the candidate cockroach repellent AI3-37135. These data are intended to provide guidance in selecting compounds for further entomological and toxicological evaluation. In addition, the data may be useful in developing preliminary safety guidelines for handling this compound.

b. Recommendations. Based on professional scientific judgment, the following recommendations are offered.

(1) AI3-37135 should be considered for more extensive entomological and toxicological testing.

(2) This compound can cause severe skin and eye damage. Personnel handling AI3-37135 should avoid contact with the skin and eyes. Protective gloves and a face shield or goggles should be worn when handling.

FOR THE COMMANDER:

N. JOE THOMPSON
Colonel, MC
Director, Occupational and Environmental Health

Encl

CF:
HQDA(DASG-PSP) (wo/encl)
Comdt, AHS (HSHA-IPM) (w/encl)
Dir, Advisory Cen on Tox, NRC (2 cy) (w/encl)
USDA, ARS (Dr. Terrence McGovern) (w/encl)
USDA, ARS - Southern Region (w/encl)
Cdr, USMRDC (SGRD-DPM/COL Reinert) (w/encl)

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO ATTENTION OF

HSHB-MO-T

PRELIMINARY ASSESSMENT OF THE RELATIVE TOXICITY OF AI3-37135 IN ANIMALS
STUDY NO. 75-51-0532-86
MARCH 1985 - JULY 1986

1. AUTHORITY.

a. Letter, US Department of Agriculture - Agricultural Research Service, Southern Region, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida, 5 December 1984.

b. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board and the Department of Agriculture, Agricultural Research, Science and Education Administration; titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

2. REFERENCES. See Appendix A for a listing of references.

3. PURPOSE. To provide preliminary toxicity data for the candidate cockroach repellent AI3-37135. This report summarizes the toxicological data for USDA candidate cockroach repellent AI3-37135. These data are intended to be used in selecting compounds for more extensive entomological and toxicological testing. The data may also be used in establishing preliminary safety guidelines for handling the material.

4. BACKGROUND.

a. General. The preliminary toxicological evaluation of candidate cockroach repellents consists of a series of acute screening tests designed to assess potential hazards from single exposures by various routes of administration. The test battery included:

(1) Rat oral approximate lethal dose (ALD).

(2) Primary irritation (skin and eye).

(3) Dermal sensitization.

Use of company names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

(4) Saturated vapor (inhalation hazard).

(5) Physiological screen.

(6) Mutagenicity (Ames test).

b. Project Information.

(1) All raw data from this study may be found in project file number 75-51-0532-86 or USAEHA Laboratory Notebooks Numbered 106, 114 and 115.

(2) In conducting the studies described in this report, the investigators adhered to the document referenced in 2a. In addition, these studies were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

c. Previous Work. Compound AI3-37135 is the last compound from the initial batch of five candidate cockroach repellents submitted to USAEHA for toxicity evaluation. References 5 through 8, Appendix A, summarize the toxicity data for the other 4 compounds in this group.

5. PROCEDURES.

a. Test Compound. Two lots (a & b) of AI3-37135 were synthesized and supplied for use in the toxicological evaluations by Dr. Terrance McGovern, USDA, Beltsville, Maryland. AI3-37135 is a pale yellow liquid. It exhibits low solubility in water but is soluble in acetone and other organic solvents. It has a molecular weight of 181 and boils at 67 °C at 0.05 mm Hg.

b. Methods.

(1) Acute Toxicity Tests. Detailed descriptions of the methodology for tests a through d listed below are published in reference 2. Methodology for the saturated vapor test is published in reference 3.

(a) Rat ALD.

(b) Skin irritancy.

(c) Eye irritancy.

(d) Dermal sensitization (Buehler technique).

(e) Saturated vapor.

(2) Mutagenicity. Mutagenicity testing was performed by Hazleton Biotechnologies Company under contract DAAD05-86-M-L723 with USAEHA. A complete description of the methodology and results may be found in the final report (reference 4).

(3) Physiological Screening. The physiological screening tests were designed to obtain basic information on the underlying mechanisms of action for this compound. Male Sprauge Dawley rats weighing between 270 - 380 gms were anesthetized with sodium pentabarbital (30 mg/kg). A heparinized cannula (15 cm length of PE50 tubing) was inserted into the left carotid artery for blood pressure monitoring. A similar catheter was inserted into the right external jugular vein for drug injection. A Statham P23-AC fluid filled pressure transducer (Gould Instruments) was used for blood pressure monitoring. The signals were processed by a Buxco Model 6 Pulmonary Function Analyzer (Buxco Electronics) and printed on a Texas Instruments® Silent 700 terminal. EKG's were monitored from LEAD II and fed through a pre-amplifier and Buxco EKG analyzer. A digital recording of wave heights and intervals was printed on a second Texas Instruments TI terminal. Following a short period of time, usually 10-15 minutes, stable physiological recordings were obtained and the animals were treated with challenge doses of standard pharmacological drugs including epinephrine, nor-epinephrine, acetylcholine and histamine. Saline injections served as a volume control. Preliminary experiments were performed in order to find optimum dosage levels. In most cases, the dosage chosen produced a marked change in blood pressure (10-50 mm Hg) lasting less than 5 minutes. Following the initial drug challenges, the test compound AI3-37135 was injected intraperitoneally, and the drug challenges were repeated 15 minutes post injection. For each drug, the maximum change from baseline condition was recorded and the pre- and post-dosing values compared. In this way, each animal served as its own control. The data were analyzed using a two-way analysis of variance with repeated measures program on an IBM® PC microcomputer. A least significant range test was used to compare pre and post-dosing values. A probability of less than 0.05 was used as the level of significance.

6. RESULTS.

a. ALD. The rat oral ALD was found to be 2222 mg/kg (Appendix B, Table B-1). This was the lowest dose that produced lethalities. Post mortem examination of the animals that died indicated hemorrhagic areas in the stomach as well as in one case, the lungs and kidneys. Animals receiving the ALD or higher dose died between 3 and 18 hours post administration.

b. Skin Irritation. Compound AI3-37135 produced a total irritancy score of 4.25 in the Draize rabbit skin irritancy test. A description of the scoring system employed in these tests is provided at Appendix C. Based on this scoring system, AI3-37135 would be considered a moderate to severe skin irritant. Heavy scurf or mild eschar formed in all cases by 3-7 days post application.

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●IBM is a registered trademark of IBM Corporation, Boca Raton, Florida.

c. Eye Irritation. Based on our Draize eye test in rabbits, this compound is a moderate eye irritant with a total irritancy score of 30.83. It produced injury to both cornea and conjunctiva; however, all but one rabbit was healed by 7 days post application. Washing the eyes with water immediately post application reduced the eye injury.

d. Skin Sensitization. Challenge doses of AI3-37135 did not produce a reaction in pretreated guinea pigs and, based on these data, it is not considered to be a sensitizer.

e. Saturated Vapor. Data from the saturated vapor test are summarized in Tables B-1 and B-2 (Appendix B). The test protocol was modified resulting in 5 animal exposure groups in two separate tests. During the initial test (control, room temperature, high temperature groups), the high temperature bubbler was held at 50 °C rather than 100 °C. In the second test, a group was exposed using a 100 °C bubbler system and there was a concurrent air-exposed group. Nominal exposure concentrations for the room temperature, 50 °C and 100 °C bubblers were 0 mg/L, 0 mg/L and 3.09 mg/L, respectively. Rats exposed to the highest concentration exhibited excessive salivation and rapid breathing, indicative of a respiratory irritant. Twenty four hours post exposure, all animals appeared normal. There were no mortalities either during the exposure or for up to 14 days post exposure.

f. Physiological Studies. Table B-3 (Appendix B) illustrates the cardiovascular effects of exposure to sublethal (0.5 x oral ALD) intraperitoneal injections of AI3-37135. The values presented represent the maximum change from resting or baseline levels in response to injections of the challenge drug. The only statistically significant change in this experiment was a decreased responsiveness to norepinephrine in repellent treated rats. Overall, there was a trend toward decreased blood pressure and heart rate in the treated rats when injected with the test drugs or in response to saline. These data suggest that AI3-37135 may exert its toxic effect in part, on the cardiovascular system.

g. Mutagenicity. AI3-37135 did not exhibit mutagenic activity under the test conditions employed. It was negative in all test strains used (Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100) and at dosages ranging from .1 µL to 25 µL per plate both activated and nonactivated test systems (reference 4).

7. CONCLUSIONS.

a. Compound AI3-37135 is moderately toxic by the oral route of exposure. It is a moderate eye irritant and a moderate to severe skin irritant. This compound presents no acute inhalation hazard at room temperatures though at higher temperatures or if the repellent is atomized as an aerosol, it may cause skin, eye and respiratory irritation. We found no indication of a sensitization reaction and it was not mutagenic in the Ames test. When administered at approximately 0.5 x the oral ALD to anesthetized, catherized rats, it produced alterations in blood pressure and heart rate which suggest the cardiovascular system as one site of potential toxic action.

b. Based on the preliminary toxicity data presented here as well as in the previous reports (Appendix A, references 5 through 8), the five candidate cockroach repellents can be ranked in priority for future developmental work. Compounds AI3-20837 and AI3-20827 exhibit the lowest potential for producing injury and have the highest priority of the group for additional testing. AI3-36161 and AI3-37135 caused more severe eye damage and for this reason, they have a slightly lower priority than the first two compounds. AI3-36174 was not recommended for additional work due to its irritancy and skin sensitizing potential.

8. RECOMMENDATIONS. The following recommendations are based on professional scientific judgment.

a. AI3-37135 should be considered for more extensive entomological and toxicological testing. Toxicological tests should include a more detailed evaluation of acute toxicity by multiple routes of administration, an assessment of the effects of repeated dosing and a complete evaluation of mutagenic potential.

b. This compound can cause severe skin and eye damage. Personnel handling AI3-37135 should avoid contact with the skin and eyes. Protective gloves and a face shield or goggles should be worn when handling.

9. ACKNOWLEDGEMENT. The project personnel shown in Appendix D assisted in the experiments.

GLENN J. LEACH
Biologist
Toxicology Division

APPROVED:

MAURICE H. WEEKS
Chief, Toxicology Division

APPENDIX A

REFERENCES

1. U.S. Department of Health and Human Services, Guide for the Care and Use of Laboratory Animals, NIH Pub No. 86-23, Revised 1985.

2. Topical Hazard Evaluation Program Procedure Guide, Toxicology Division, US Army Environmental Hygiene Agency (USAEHA), October 1985.

3. Standing Operating Procedures, HSHB-OT, Toxicology Division, USAEHA.

4. Final Report, Mutagenicity Evaluation of AI3-37135B in the Ames Salmonella/Microsome Reverse Mutation Assay, Hazleton Biotechnologies Company, HBC Project No. 20988, August 1986.

5. Report, USAEHA, Preliminary Assessment of the Relative Toxicity of AI3-20837 in Animals Study No. 75-51-0528-86, March 1985 - July 1986.

6. Report, USAEHA, Preliminary Assessment of the Relative Toxicity of AI3-20827 in Animals Study No. 75-51-0529-86, March 1985 - July 1986.

7. Report, USAEHA, Preliminary Assessment of the Relative Toxicity of AI3-36161 in Animals Study No. 75-51-0530-86, March 1985 - July 1986.

8. Report, USAEHA, Preliminary Assessment of the Relative Toxicity of AI3-36174 in Animals Study No. 75-51-0531-86, March 1985 - July 1986.

Study No. 75-51-0532-86, March 1985 - July 1986

APPENDIX B

RESULTS

TABLE A-1. SUMMARY OF TOXICITY DATA CANDIDATE COCKROACH REPELLENT AI3-20837

ALD (Mg/Kg)	Skin Category	Eye Category	Sensitization	Sat Vapor	Physio	Ames
2222	IV	E	Negative	No deaths High conc-irritant	Slight decrease Blood Pressure Heart Rate	Negative

* Cardiovascular - Parameters monitored included blood pressure, heart rate and electrocardiogram in anesthetized rats.

TABLE B-2. SUMMARY OF SATURATED VAPOR RESULTS COMPOUND AI3-37135

Parameter/ Test Group	BW (grams)	$\frac{LW}{BW} \times 100$	$\frac{KW}{BW} \times 100$	$\frac{HW}{BW} \times 100$	$\frac{LGW}{BW} \times 100$	$\frac{TW}{BW} \times 100$	$\frac{BRW}{BW} \times 100$	$\frac{SW}{BW} \times 100$
Control	255 ± 7	5.727 ± 0.107	1.080 ± 0.034	0.454 ± 0.012	0.633 ± 0.021	0.949 ± 0.028	0.748 ± 0.021	0.335 ± 0.020
Room Temp	258 ± 5	5.855 ± 0.110	1.030 ± 0.015	0.485 ± 0.634	0.652 ± 0.351	0.932 ± 0.039	0.704 ± 0.016	0.332 ± 0.014
50 °C	259 ± 6	5.811 ± 0.181	1.088 ± 0.038	0.474 ± 0.013	0.625 ± 0.050	0.938 ± 0.048	0.709 ± 0.014	0.333 ± 0.019
Control	233 ± 3	5.825 ± 0.207	1.117 ± 0.029	0.467 ± 0.025	0.564 ± 0.065	0.915 ± 0.025	0.792 ± 0.009	0.347 ± 0.052
100 °C	228 ± 6	5.689 ± 0.104	1.125 ± 0.041	0.467 ± 0.008	0.699 ± 0.030	0.953 ± 0.358	0.810 ± 0.027	0.419 ± 0.025

Body weight (BW) and organ to body weight ratios, saturated vapor test, compound AI3-20837. Organ weights abbreviated as follows: Liver weight (LW), kidney weight (KW), heart weight (HW), lung weight (LGW), testes weight (TW), brain weight (BRW), spleen weight (SW). Numbers presented represent the mean ± standard error of the mean for six animals. There were no statistically significant differences among the three groups in any of the measured parameters.

TABLE B-3. SUMMARY OF PHYSIOLOGICAL DATA, COMPOUND AI3-37135

	BP		HR		QRS		QT		PW		RW		PH		RH	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
EPI	171 ± 6	149 ± 9	374 ± 57	326 ± 54	28 ± 1	31 ± 3	59 ± 1	62 ± 3	20 ± 2	24 ± 2	16 ± 1	17 ± 1	0.07 ± 0.02	0.13 ± 0.02	0.83 ± 0.15	0.87 ± 0.10
ACH	102 ± 7	69 ± 5	416 ± 13	340 ± 31	27 ± 1	30 ± 2	56 ± 2	58 ± 2	23 ± 2	23 ± 2	15 ± 1	17 ± 1	0.09 ± 0.01	0.10 ± 0.01	0.75 ± 0.12	0.69 ± 0.11
NE	157 ± 6	104 ± 8*	354 ± 31	354 ± 31	28 ± 1	29 ± 2	59 ± 1	59 ± 2	22 ± 1	22 ± 1	15 ± 1	17 ± 1	0.10 ± 0.02	0.12 ± 0.01	0.77 ± 0.15	0.66 ± 0.12
HIST	105 ± 4	64 ± 3	406 ± 12	301 ± 19	28 ± 1	31 ± 3	57 ± 1	61 ± 3	23 ± 2	18 ± 4	16 ± 1	17 ± 1	0.11 ± 0.02	0.07 ± 0.03	0.71 ± 0.11	0.67 ± 0.10
SAL	122 ± 6	79 ± 3	394 ± 9	319 ± 18	28 ± 2	31 ± 2	59 ± 1	60 ± 2	23 ± 2	19 ± 5	16 ± 1	17 ± 1	0.10 ± 0.02	0.08 ± 0.03	0.76 ± 0.11	0.68 ± 0.10

Values presented are the mean ± the standard error of the mean for five animals. Maximum changes in response to drug challenges were recorded. Data were analyzed with a two-way analysis of variance for repeated values. Table abbreviations are as follows: BP - blood pressure (mm HG), HR - heart rate (beats/min), WRS - width of QRS complex (msec), PW - P width (msec), RW - R - width (msec), PH - P height (millivolts), RH - R height (millivolts), PRE - pre treatment response, Post - response to drug challenge 15 min post injection, EPI - epinephrine, ACH - acetylcholine, NE - nor-epinephrine, HIST - histamine, SAL - saline. *Indicates significant difference when compared to pre exposure results. ($P < 0.05$).

APPENDIX C

DEFINITIONS OF CATEGORIES OF SKIN AND EYE IRRITANTS

1. Skin irritants.

a. Category I - Compounds producing no irritation of intact skin or no greater than mild primary irritation of the skin surrounding an abrasion.

b. Category II - Compounds producing mild primary irritation of the intact skin and the skin surrounding an abrasion.

c. Category III - Compounds producing moderate primary irritation of the intact skin and the skin surrounding an abrasion.

d. Category IV - Compounds producing moderate to severe primary irritation of the intact skin and of the skin surrounding an abrasion and in addition, producing necrosis, vesiculation, and/or eschars.

e. Category V - Compounds impossible to classify because of staining of the skin or other masking effects owing to physical properties of the compound.

2. Eye irritants.

a. Category A - Compounds noninjurious to the eye.

b. Category B - Compounds producing mild injury to the cornea.

c. Category C - Compounds producing mild injury to the cornea and in addition some injury to the conjunctiva.

d. Category D - Compounds producing moderate injury to the cornea.

e. Category E - Compounds producing moderate injury to the cornea and in addition, some injury to the conjunctiva.

f. Category F - Compounds producing severe injury to the cornea and to the conjunctiva.

APPENDIX D

PROJECT PERSONNEL

The experiments described in this report were performed by a multidisciplinary group under the direction of Glenn Leach. The group included the following:

1. Lynn M. Balczewski, SGT, Lab Animal Care Specialist.
2. John G. Harvey, Bio Lab Tech.
3. John T. Houpt, Bio Lab Tech.
4. R. David Russell, CPT, VC.

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